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Zebra Mussel Research Program

Studies of Heat Tolerance of Zebra Mussels: Effects of Temperature Acclimation and Chronic Exposure to Lethal Temperatures

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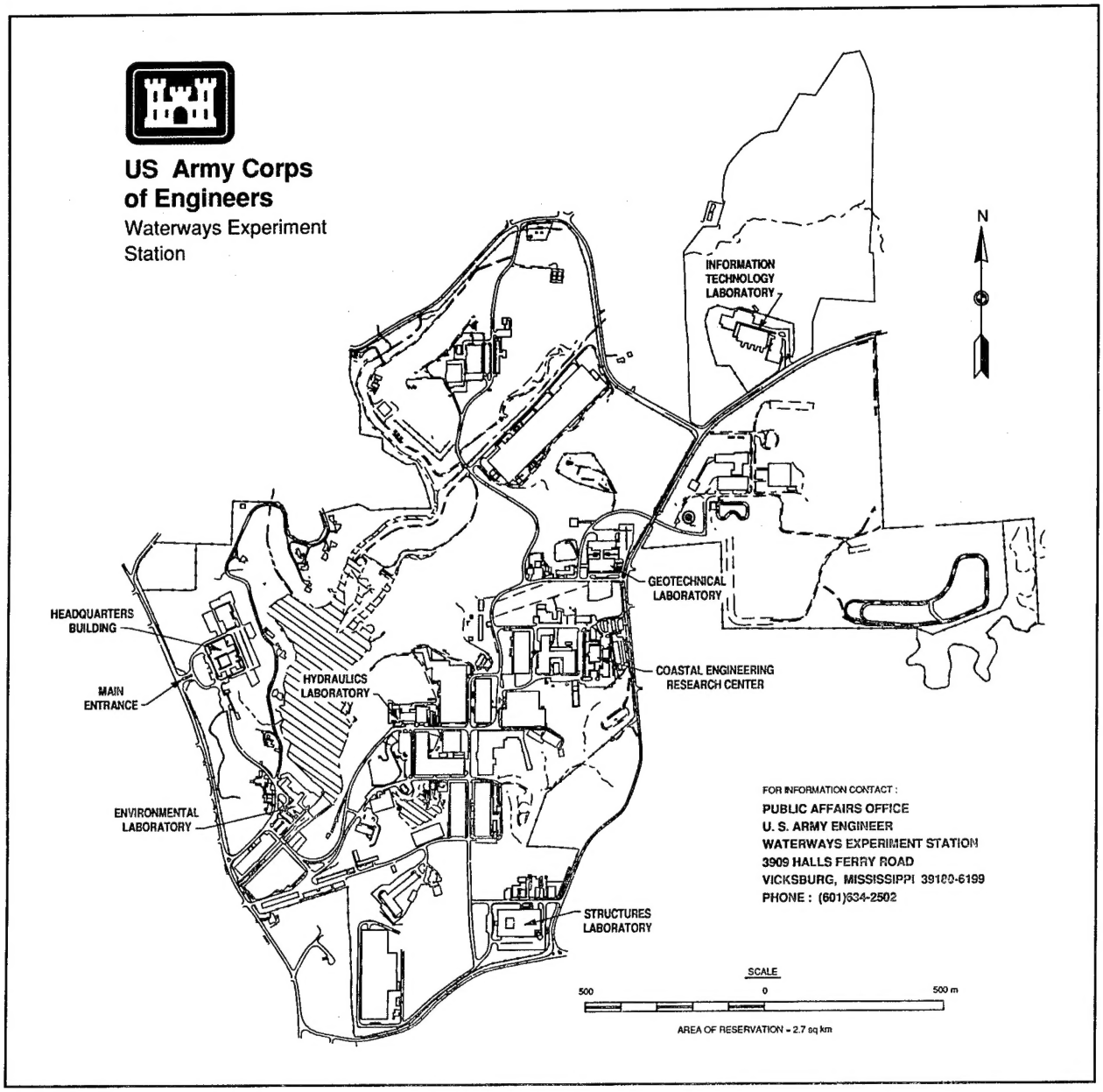

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Contents

Preface

The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 specified that the Assistant Secretary of the Army, Civil Works, will develop a program of research and technology development for the environmentally sound control of zebra mussels (*Dreissena polymorpha*). As a result, the U.S. Army Engineer Waterways Experiment Station (WES) initiated a program to develop control strategies for this species.

This report was prepared by Dr. Robert F. McMahon, Mr. Milton A. Matthews, Mr. Thomas A. Ussery, Mr. R. Chase, and Mr. Michael Clarke, Center for Biological Macrofouling Research, University of Texas at Arlington, Arlington, TX. Mr. Gary L. Dye, Lockmaster of the U.S. Army Corps of Engineers Black Rock Lock, in Buffalo, New York, collected and shipped mussels to the Center for Biological Macrofouling Research. Research for this report was funded under Contract DACW39-92-K-0004 with WES. Drs. Andrew C. Miller and Barry S. Payne, Environmental Laboratory (EL), WES, managed the contract for WES. Dr. Edwin A. Theriot, EL, was Program Manager of the Zebra Mussel Research Program.

During the conduct of this study, Dr. Theriot was Chief, Aquatic Ecology Branch; Dr. Conrad J. Kirby was Chief, Ecological Research Division; and Dr. John W. Keeley was Director, WES.

Dr. Robert W. Whalin was Director of WES at the time of publication of this report. COL Bruce K. Howard, EN, was Commander.

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1 Introduction

Thermal treatment is an accepted nonchemical mitigation technology for control of raw water system macrofouling by both marine and freshwater bivalves (Electric Power Research Institute 1984; Stock and Del La Parra 1983) and has been utilized both in Europe and the United States to mitigate zebra mussel, *Dreissena polymorpha* (Pallas), fouling, especially in steam-electric power stations in which raw water system temperatures can be elevated to lethal levels by partial recirculation of heated discharge waters (Electric Power Research Institute 1992; Claudi and Mackie 1993; Jenner and JanssenMommen 1992; Mackie et al. 1989)..

For *D. polymorpha* and other macrofouling species, the upper lethal thermal limits on which thermal mitigation strategies are based have generally been determined as either "acute upper lethal temperatures" or "chronic or incipient upper lethal temperatures." Acute upper lethal temperatures are measured as the temperature at which death occurs when water temperature is raised at a specific rate. The results of testing for acute upper lethal temperatures yield the mean lethal temperature, LT_{50} or LT_{100} values (i.e., estimated temperature for 50-percent or near 100-percent sample mortality estimated by probit analysis (Bliss 1936)) or SM_{100} values (i.e., the actual recorded temperature of 100-percent sample mortality) (McMahon et al. 1993; Stirling 1982). Use of acute upper lethal temperature treatment to mitigate zebra mussel fouling would be most applicable in raw water systems where it is difficult to maintain lethal temperatures for extended periods. In these systems, it is more practical to increase water temperature to a level which induces an instantaneous or "acute" 100-percent mussel mortality followed by return to normal operating temperatures (McMahon et al. 1993). As acute thermal treatment does not require precise, long-term regulation of elevated temperatures, it has been proposed for use in zebra mussel mitigation in raw water systems where operation above normal water temperatures for prolonged periods reduces efficiency and increases component wear, making chronic thermal treatment of zebra mussels economically unfeasible. Acute thermal mitigation may also be particularly applicable for use in off-line components such as intake embayments heated by steam injection (Kovalak 1993) or in various isolated sections or components of mussel-fouled raw water systems warmed by steam injection or other means (Miller et al. 1992).

In contrast to zebra mussel thermal mitigation strategies based on acute upper thermal limits, strategies based on "chronic or incipient upper thermal limits" involve continuous exposure of zebra mussel infestations to constant lethal temperatures for periods of long-enough duration to achieve significant mortality. The laboratory studies on which chronic thermal mitigation treatments are based yield estimates of the period of time over which a sample of mussels can tolerate continuous exposure to specific upper lethal temperatures. This type of temperature tolerance testing involves long-term holding of test individuals at a specific acclimation temperature, followed by instantaneous or near instantaneous transfer into a series of constantly maintained lethal test temperatures and recording survival times. The results of such testing are generally expressed as the mean time to death at a specific temperature, LT_{50} or LT_{100} values (i.e., the estimated time required for induction of 50-percent or near 100-percent sample mortality estimated by probit analysis [Bliss, 1936]) or SM_{100} values (i.e., the actual exposure time required to induce 100-percent sample mortality at a particular lethal temperature) (Iwanyzki and McCauley 1992; Stirling 1982; Stock and Del La Parra 1983). Chronic thermal treatment for mitigation of zebra mussel infestations is most applicable in industrial and steam-electric power station raw water systems which generate heated discharge water and are designed to recirculate or backwash heated effluents into their intakes in order to maintain operating temperatures at relatively constant, elevated, lethal levels for prolonged periods.

Many industrial and power station raw water systems, particularly in the northern latitudes of North America, have been designed for such recirculation of heated discharge water to prevent winter freezing of water or formation of frazzle ice within their raw water systems (Claudi and Mackie 1993; Electric Power Research Institute 1992; Neuhauser et al. 1993), giving them the capability for chronic thermal mitigation of zebra mussel infestations. Chronic thermal mitigation of zebra mussel infestations is commonly employed in Europe (Jenner and Janssen-Mommen 1992) and has begun to be utilized in North America (Neuhauser et al. 1993).

The main advantage of chronic thermal mitigation strategies is that the water temperatures required for zebra mussel mitigation are generally lower (Claudi and Mackie 1993; Electric Power Research Institute 1992; Iwanyzki and McCauley 1992; Jenner and Janssen-Mommen 1992; Neuhauser et al. 1993) than those required for mitigation by acute thermal treatment (McMahon et al. 1993). As the discharge temperatures of many industries and power stations are regulated by state and federal environmental agencies (Electric Power Research Institute 1992; Neuhauser et al. 1993), the higher discharge temperatures required for acute thermal mitigation treatment may not be permitted by these agencies, especially in systems such as power stations with once-through condenser water systems that discharge large volumes of heated water.

The acute upper lethal temperature of zebra mussels is affected by both their prior temperature experience (e.g., the "acclimation" temperature or operating water temperature prior to thermal treatment) and the rate at which

temperature rises to the acute temperature inducing instantaneous death. The temperature at which instantaneous death ensues increases with increased acclimation temperature and increased heating rate (McMahon et al. 1993). Experimental data have been utilized to develop mathematical models predicting the acute upper lethal temperatures of zebra mussels based on acclimation temperature and heating rate (McMahon et al. 1993). These models allow development of acute thermal mitigation strategies for zebra mussel fouling on a "site-specific" basis, predicting the temperature which must be exceeded for 100-percent mussel kill based on previous operating temperature and the rate at which system water temperature can be heated. Similarly, acclimation temperature and treatment temperature have been shown to affect the exposure time required to kill zebra mussels under chronic thermal mitigation treatment with required exposure time increasing with increased acclimation temperature and decreased treatment temperature (Jenner and Janssen-Mommen 1992; Iwanyzki and McCauley 1992, and citations therein). However, a mathematical model has yet to be developed which will allow prediction of the required exposure time for mitigation of a zebra mussel infestation based on its prior operating (i.e., acclimation) temperature experience and the specific lethal treatment temperature applied to the system. Such a model could be of great value in designing a site-specific chronic thermal treatment strategy for mitigation of zebra mussel fouling, incorporating prior intake water temperatures, temperature of thermal treatment, and maximum discharge temperatures permitted by regulatory agencies. Such a model would also assist a facility in the decision to utilize either "acute" or "chronic" treatment for thermal mitigation of a zebra mussel infestation.

This report presents a laboratory study of the effects of both prior acclimation temperature and exposure temperature on the chronic upper thermal tolerance times of zebra mussels. The resulting data are utilized to develop a simple mathematical model predicting the chronic exposure time required to induce death based on prior acclimation and exposure temperatures. This model can be utilized for development of site-specific chronic thermal mitigation strategies along with that previously developed for acute thermal treatment strategies (McMahon et al. 1993), providing a comprehensive means for evaluating the efficacy of thermal treatment for zebra mussel fouling and the most appropriate thermal treatment strategy on a site-specific basis.

2 Materials and Methods

Zebra mussels were collected from the vertical sides of a concrete guide wall at the U.S. Army Corps of Engineers, Black Rock Navigation Lock on the Niagara River in Buffalo, NY. Immediately following collection, mussels were shipped overnight in insulated, cooled containers to the Center for Biological Macrofouling Research at the University of Texas at Arlington, where they were maintained in a 200 ℓ (75 gal) refrigerated "Living Stream" holding tank at a constant temperature of 5 °C (41 °F) without feeding in dechlorinated City of Arlington tap water. All mussels were utilized in experiments within two months of collection. The metabolic rate of zebra mussels held at 5 °C is so greatly depressed that significant reduction in dry tissue mass cannot be detected within a 60-day holding period (Chase and McMahon 1994). Thus, mussels utilized in the experiments were in good physiological condition.

Groups of greater than 30 mussels were removed from the holding tank and placed in 9-cm-diam by 5-cm-high glass crystallization dishes covered with a 1-mm mesh nylon screen held in place with a rubber band, preventing mussel escape. The crystallization dishes were transferred into plastic holding tanks (22 cm deep by 21 cm wide by 31 cm long) containing 17 ℓ (4.5 gal) of dechlorinated tap water. Holding tanks were held in refrigerated incubators in which five groups of mussels were acclimated to constant temperatures of 10, 15, 20, 25, or 30 °C (50, 59, 68, 77, or 86 °F) (±0.5 °C) for a period of greater than 14 days prior to determination of chronic lethal temperature tolerance times. A sixth group of mussels acclimated to 5 °C (41 °F) was similarly held in a plastic aquarium placed in the 200-ℓ, 5 °C "Living Stream" holding tank. The acclimation tanks medium was replaced every three days with dechlorinated tap water preheated or precooled to the temperature of acclimation.

There was little, if any, mortality observed in mussels held at any of the acclimation temperatures. Only mussels which had made byssal attachment to the walls of the crystallization dishes or to the shells of other mussels during the acclimation period were utilized in determination of chronic thermal tolerance. Unattached mussels were removed from the dishes prior to experimentation. In dishes with more than 30 attached mussels, individuals were randomly culled to sample sizes of approximately 30 mussels just prior to experimentation. Sample size at any one acclimation/test temperature

combination ranged from 26 to 33. Only byssally attached mussels were utilized in experiments because removal from the byssus has been demonstrated to reduce the tolerance of zebra mussels to stress, such as that induced by biocide exposure (McMahon, Shipman, and Long 1992).

After temperature acclimation, groups of mussels in crystallization dishes for each of the six acclimation temperatures were submerged separately in 25-cm-deep by 22-cm-wide by 43-cm-long insulated water baths containing 23 ℓ of dechlorinated tap water constantly cooled by a Forma Scientific, Refrigerated Cold Finger (Model 2535). Water in the baths was circulated and initially held at 5 °C by a Haake D1 Water Bath Temperature Regulator. One group of 5 °C-acclimated individuals was placed in each bath and the water temperature raised 1 °C every 10 min. Dishes containing samples of the other acclimation groups (10, 15, 20, 25, and 30 °C) were placed in the bath at the point when rising bath temperature corresponded to their acclimation temperature, thus avoiding temperature shock to any test group. After all acclimation groups had been placed in water baths, bath temperature was increased at 1 °C/10 min to final test temperatures of 31, 32, 33, 34, 35, 36, or 37 °C (87.8, 89.6, 91.4, 93.2, 95.0, 96.8, or 98.6 °F), where they were then held constant ($\pm$0.1 °C) by temperature regulators. Starting times for raising water temperatures in the baths were staggered so that all baths reached their respective final test temperatures at the same time. Rapid water circulation by the regulators ensured uniform temperature and oxygenation throughout the baths.

Throughout thermal tolerance time determinations, bath water temperature was monitored with a fast-responding micro-thermistor and a Model 43-DT, Yellow Springs Instrument Company Tele-Thermometer. Every 3 days throughout the course of the experiment, samples of mussels were transferred to water baths containing fresh media at the appropriate test temperature to prevent media contamination with the mussel's metabolic end products.

The thermal tolerance times of mussels held at each test temperature were determined by periodically removing crystallization dishes and observing the contained mussel samples for mortality as indicated by widely gaping valves. The viability of mussels with gaping valves was determined by gentle touching of the tissues of the posterior mantle edge or siphons with the bristles of a fine brush. If this tactile stimulation did not elicit a valve closure response, the mantle edges and siphons were more vigorously probed with the hard, pointed end of the brush handle. If this more vigorous tactile stimulation still did not elicit valve closure, the individual was considered to be dead. A previous study had indicated that thermally stressed mussels which displayed widely gaping valves did not regain capacity to close their valves after 12 hr recovery at room temperature and thus were considered dead (McMahon et al. 1993). Dead mussels were removed from the crystallization chambers, their times of death recorded, and their shell lengths (SL, the linear distance between the posterior margin of the shell and the anterior tip of the umbos) measured to the nearest 0.1 mm with dial calipers. The size range of all mussels utilized in the experiment was 11.0 to 35.4 mm with mean size being 20.2 mm

(n = 1259, s.d = ± 3.3). After baths reached test temperatures, mortality in mussel samples was monitored every 15 min for the first 2 hr, every 30 min for the next 3 to 5 hr, every hour for the next 17 to 21 hr, and every 3 to 4 hr thereafter, until all mussels in all acclimation groups at all test temperatures had died.

3 Results

Mean times to death were determined for each acclimation group at each test temperature (Table 1). The time required for 50-percent sample mortality (LT_{50}) and near 100-percent sample mortality (LT_{100}) was estimated from cumulative percent mortality values for each acclimation/test temperature sample by Probit Analysis (Bliss 1936). In addition, the actual time required to achieve 100-percent sample mortality was recorded (Table 1). Mean thermal tolerance times, LT_{50} values, and SM_{100} values increased exponentially with both increasing acclimation temperature and decreasing test temperature (Figure 1); thus, in all statistical testing the natural logarithm (ln) of tolerance time was utilized to linearize the data.

Least squares multiple linear regression analysis relating time to death in individual mussels as the dependent variable to acclimation temperature, test temperature, and SL as independent variables indicated that all three variables significantly affected thermal tolerance ($n = 1259$, $r = 0.932$, $F = 2757$, $P < 0.00001$) (Table 2). The very high correlation coefficient (R^2) of this multiple regression indicated that the effects of these three variables accounted for 87 percent of all recorded variation in thermal tolerance times. Such a high level of correlation of tolerance time with treatment variables suggested that there was little or no effect of either sample holding chambers or water baths on tolerance times, allowing statistical analysis of the data to utilize individual mussels as the experimental unit.

Thermal tolerance times were found to increase significantly with increased acclimation temperature ($P < 0.0001$), and to decrease significantly with increased test temperature ($P < 0.0001$) and increased individual SL ($P < 0.0001$) (Table 2). The correlation coefficients for these variables indicated that test temperature had the greatest effect on thermal tolerance time, with acclimation temperature and SL having relatively similar but lesser effects over the tolerated acclimation temperature range (0 to 30 °C) and typical SL range of North American zebra mussels (1 to 35 mm). Multiple least squares linear regressions also indicated that natural logarithms of LT_{50}, LT_{100}, and SM_{100} values were similarly correlated to the independent variables of acclimation temperature and test temperature ($n = 42$, $r = 0.949$-0.969, $F = 185$-294, $P < 0.00001$) (Tables 3, 4, 5). Tolerance times are presented for exposures to lethal temperatures of 31, 32, 33, 34, 35, 36, and 37 °C.

Table 1
Effect of Temperature Acclimation on Temperature Tolerance Times of *Dreissena polymorpha* on Exposure to Different Lethal Test Temperatures Expressed as Means, LT_{50} Values (Estimated time for 50-percent sample mortality), LT_{100} Values (Estimated time for 100-percent sample mortality), and SM_{100} Values (Actual time for 100-percent mortality)

Acc. Temp., °C	Test Temp. °C	n	Mean Hours to Death (±S.E.)	LT_{50} °C	LT_{100} °C	SM_{100}	Range
5	31	32	148.15 ± 18.93	124.93	1,601.391	399.70	8.7 - 399.7
	32	33	25.17 ± 2.37	21.95	01.44	54.50	4.5 - 54.5
	33	33	7.45 ± 0.37	.83	12.79	10.30	4.3 - 10.3
	34	29	2.74 ± 0.21	1.87	5.88	5.50	1.5 - 5.5
	35	28	1.62 ± 0.13	1.13	3.23	3.33	1.33 - 3.33
	36	30	1.17 ± <0.0001	0.59	1.17	1.17	1.17 - 1.17
	37	26	1.0 ± <0.0001	0.50	1.00	1.00	1.17 - 1.17
10	31	30	292.58 ± 55.40	203.62	5,189.83	1,040.21	15.67 - 1,040.16
	32	30	23.09 ± 3.73	2.11	186.83	15.99	6.83 - 115.99
	33	31	6.85 ± 0.46	6.39	15.56	12.34	3.17 - 12.34
	34	29	1.66 ± 0.18	1.77	8.05	5.50	1.0 - 5.50
	35	30	0.94 ± 0.04	0.70	1.59	1.25	0.50 - 1.25
	36	30	0.47 ± 0.02	0.39	0.09	0.50	0.25 - 0.50
	37	31	0.46 ± 0.02	0.40	0.10	0.50	0.25 - 0.50
15	31	26	340.25 ± 56.26	250.32	5,423.98	1,081.66	12.67 - 1,081.66
	32	30	20.84 ± 2.46	0.55	87.95	7.99	7.83- 67.99
	33	29	8.58 ± 0.83	8.34	36.44	23.34	4.17 - 23.34
	34	30	2.91 ± 0.21	2.43	7.50	5.00	1.25 - 5.00
	35	31	1.06 ± 0.10	1.11	4.62	4.00	0.50 - 4.00
	36	30	0.52 ± 0.01	0.44	0.73	0.75	0.50 - 0.75
	37	30	0.52 ± 0.02	0.45	0.75	0.75	0.25 - 0.75
20	31	32	203.53 ± 22.53	186.54	1,308.46	471.70	38.7 - 471.7
	32	33	56.38 ± 4.19	9.60	208.58	110.50	10.5 - 110.5
	33	33	14.48 ± 1.17	11.81	44.23	30.30	4.3 - 30.3
	34	30	5.50 ± 0.33	4.76	13.37	11.50	3.5 - 11.5
	35	30	3.26 ± 0.07	1.76	3.23	3.33	1.33 - 3.33
	36	30	1.17 ± <0.0001	0.59	1.17	1.17	1.17 - 1.17
	37	30	1.0 ± <0.0001	0.50	1.00	1.00	1.00 -1.00
25	31	31	527.10 ± 50.17	456.16	3,449.07	1,129.42	68.16 - 1,129.41
	32	28	72.90 ± 10.01	0.54	654.25	75.99	18.50 - 275.99
	33	30	12.32 ± 0.91	11.97	35.11	23.34	5.67 - 23.34
	34	28	5.88 ± 0.50	5.55	17.06	12.17	3.50 - 12.17
	35	30	3.75 ± 0.21	2.79	12.39	5.00	1.00 - 5.00
	36	31	1.04 ± 0.06	0.79	2.16	1.50	0.50 - 1.50
	37	30	0.84 ± 0.04	0.66	1.48	1.25	0.50 - 1.25
30	31	29	390.67 ± 32.98	333.41	2,384.84	769.99	32.17 - 769.99
	32	29	162.49 ± 19.69	42.367	993.19	399.99	32.00 - 399.99
	33	28	73.43 ± 5.82	.64	209.46	13.99	29.00 - 139.99
	34	29	14.77 ± 1.13	12.17	72.58	23.17	2.50 - 23.17
	35	30	5.21 ± 0.38	4.04	31.60	8.50	0.50 - 8.50
	36	30	1.82 ± 0.14	1.49	5.17	3.00	0.50 - 3.00
	37	30	0.84 ± 0.04	0.89	3.40	1.25	0.25 - 1.25

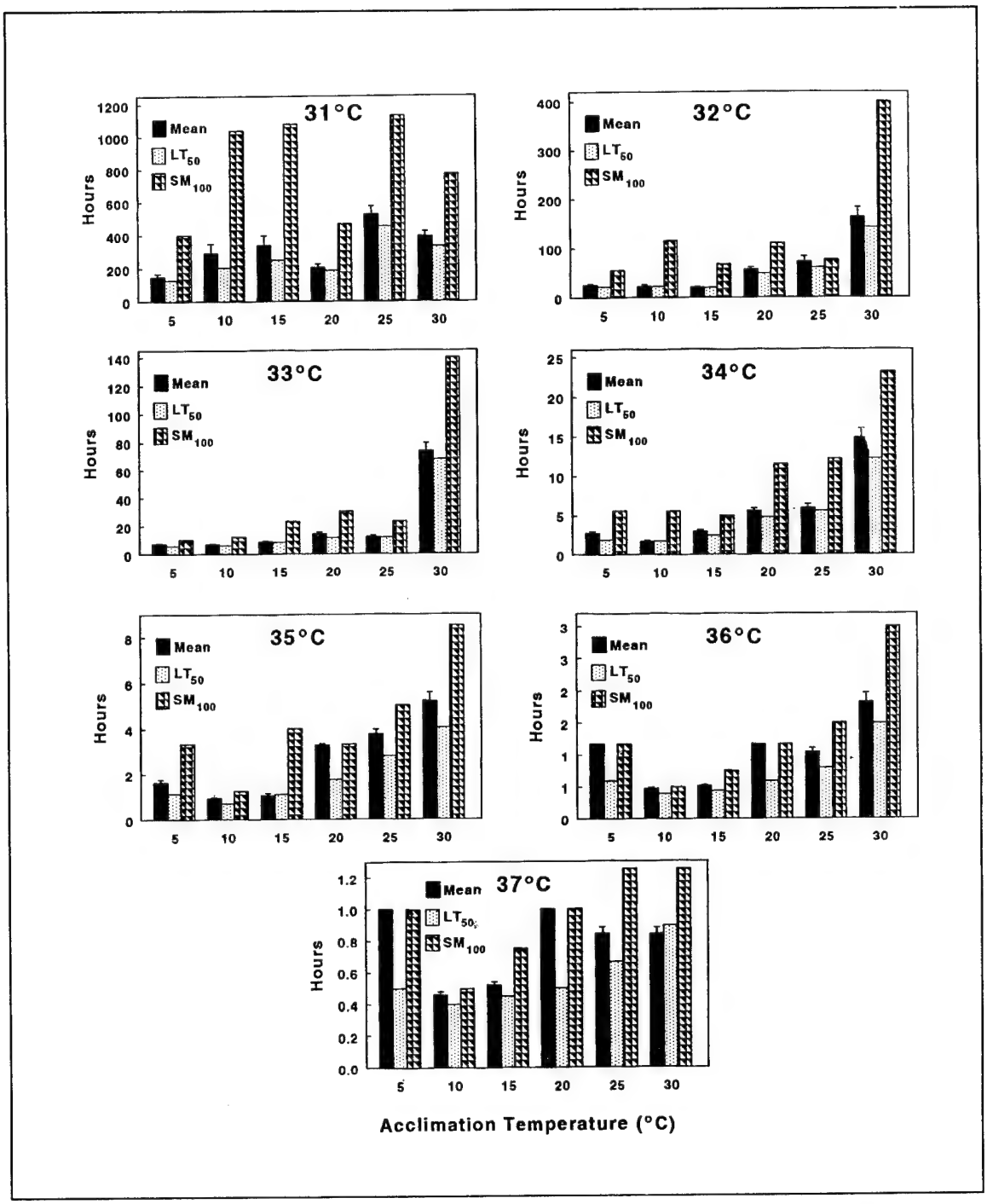

Figure 1. Chronic (incipient) upper lethal thermal tolerance times in zebra mussels, *Dreissena polymorpha* from the Niagara River. Vertical axis in all graphs is tolerance of lethal temperatures measured in hours as mean tolerance times (solid histograms), LT_{50} values, the estimated time for 50-percent sample mortality (stippled histograms) and SM_{100} values, the actual time for 100-percent sample mortality (checkered histograms). The horizontal axis in all graphs is acclimation temperature in °C. Vertical bars atop histograms are standard errors of the means. Tolerance times are presented for exposures to lethal temperatures of 31, 32, 33, 34, 35, 36, and 37 °C

Table 2
Model Fitting Results and Coefficients for a Multiple Linear Regression Analysis Relating the Natural Logarithm of Time to Death (hr) in specimens of *Dreissena polymorpha* (Dependent Variable) to Acclimation Temperature (°C), Test Temperature (°C), and Shell Length (mm) (Independent Variables)

Indpend. Variable	Coefficient	Standard Error	T-value	Probability	Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	Probability
Constant	33.982	0.391	86.90	<0.00001[1]	Model	4,697.09	3	1,565.7	2,756.73	<0.00001[1]
Acclimation Temperature	0.0599	0.00255	22.73	<0.00001[1]	Error	712.78	1,255	0.567		
Test Temperature	-0.944	0.0106	-88.67	<0.00001[1]	Total	5,409.87	1,258			
Shell Length	-0.0517	0.00656	-7.89	<0.00001[1]						

[1] Significant difference at $P \leq 0.05$. Regression Coefficient (r) = 0.932.

Table 3
Model Fitting Results and Coefficients for a Multiple Linear Regression Analysis Relating the Natural Logarithm of Estimated Time to Death of 50 Percent of the Sample (LT_{50}) (Dependent Variable) in Specimens of *Dreissena polymorpha* to Acclimation Temperature (°C) and Test Temperature (°C) (Independent Variables)

Indpend. Variable	Coefficient	Standard Error	T-value	Probability	Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	Probability
Constant	35.831	1.480	23.91	<0.00001[1]	Model	184.42	2	92.21	249.96	<0.00001[1]
Acclimation Temperature	0.0578	0.0101	5.72	<0.00001[1]	Error	12.19	39	0.0313		
Test Temperature	-1.018	0.0431	-23.61	<0.00001[1]	Total	196.62	41			

[1] Significant difference at $P \leq 0.05$. Regression Coefficient (r) = 0.967.

Table 4
Model Fitting Results and Coefficients for a Multiple Linear Regression Analysis Relating the Natural Logarithm of Estimated Time to Death of Nearly 100 Percent of the Sample (LT_{100}) (Dependent Variable) in Specimens of *Dreissena polymorpha* to Acclimation Temperature (°C) and Test Temperature (°C) (Independent Variables)

Indpend. Variable	Coefficient	Standard Error	T-value	Probability	Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	Probability
Constant	46.817	2.442	19.17	<0.00001[1]	Model	315.50	2	157.7	185.30	<0.00001[1]
Acclimation Temperature	0.0788	0.0167	4.73	<0.00001[1]	Error	33.20	39	0.851		
Test Temperature	-1.328	0.0712	-18.66	<0.00001[1]	Total	348.70	41			

[1] Significant difference at $P \leq 0.05$. Regression Coefficient (r) = 0.949.

Table 5
Model Fitting Results and Coefficients for a Multiple Linear Regression Analysis Relating the Natural Logarithm of Actual Time to Death of 100 Percent of the Sample (SM_{100}) (Dependent Variable) in Specimens of *Dreissena polymorpha* to Acclimation Temperature (°C) and Test Temperature (°C) (Independent Variables)

Indpend. Variable	Coefficient	Standard Error	T-value	Probability	Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	Probability
Constant	40.002	1.622	24.65	<0.00001[1]	Model	221.17	2	110.59	294.26	<0.00001[1]
Acclimation Temperature	0.0514	0.0111	4.63	<0.00001[1]	Error	14.66	39	0.376		
Test Temperature	-1.126	0.0473	-23.81	<0.00001[1]	Total	235.81	41			

[1] Significant difference at $P \leq 0.05$. Regression Coefficient (r) = 0.969.

Multiple linear regression equations allowing prediction of ln mean thermal tolerance time (hr) based on acclimation temperature (°C), test temperature (°C), and shell length (mm) and prediction of ln LT_{50}, ln LT_{100}, or ln SM_{100} values (hr) based on acclimation and test temperatures are displayed in Table 6 along with corresponding regression parameters. When the mean thermal tolerance times predicted by these equations (mean thermal tolerance computed for a mussel with an SL of 15 mm) were graphically expressed against different treatment temperatures at different acclimation temperatures, thermal tolerance time was shown to decline exponentially with increasing treatment temperature and decreasing acclimation temperature (Figures 2A-D). Whether expressed as absolute tolerance times (Figure 2A), LT_{50} (Figure 2B), LT_{100} (Figure 2C), or SM_{100} values (Figure 2D), mussel death at treatment temperatures of 37 °C and above was nearly instantaneous, occurring within less than 1 hr regardless of prior acclimation temperature experience. Regression analysis (Figure 2A) indicated that, below 37 °C, thermal tolerance times increased exponentially with decreased test temperatures and were greatly affected by acclimation temperature. Thus, at 34 °C, the estimated mean tolerance times for a 15-mm-SL mussel ranged from 4 hr in 5 °C-acclimated individuals to 17 hr in 30 °C-acclimated individuals (Figure 2A) while estimated SM_{100} values over the corresponding acclimation temperature range ranged from 5.6 to 26.1 hr (Figure 2D). At treatment temperatures below 34 °C, the exponential relationship with treatment and acclimation temperatures greatly extended thermal tolerance times. At 31 °C (the lowest temperature lethal to the mussels tested), mean tolerance time in a 15-mm-SL individual was estimated to be 69 hr if acclimated to 5 °C, rising to 293 hr if acclimated to 30 °C (Figure 2A). The range of corresponding SM_{100} values at a 31 °C-treatment temperature increased to 211 hr to 737 hr over a 5 to 30 °C-acclimation temperature range (Figure 2D).

Table 6
Multiple Least Squares Linear Regression Equations Relating the Natural Logarithm of Thermal Tolerance Time (Hours) Expressed as either Mean Tolerance Times, LT_{50} (Estimated Time for 50-Percent Sample Mortality), LT_{100} (Estimated Time for Near 100-Percent Sample Mortality), or SM_{100} (Actual Time for 100-Percent Sample Mortality) (Dependent Variable) to Acclimation Temperature (AT in °C), Test Temperature (TT in °C) and Specimen Shell Length (SL in mm) (Independent Variables)

ln Mean Hrs = 33.982 + 0.0579(AT in °C) - 0.944(TT in °C) - 0.0517(SL in mm) n = 1,260, F = 2,756, r = 0.932, P < 0.00001**
ln LT_{50} in Hrs = 35.381 + 0.0578(AT in °C) - 1.018(TT in °C) n = 42, F = 295, r = 0.967, P 0.00001**
ln LT_{100} in Hrs = 46.817 + 0.0788(AT in °C) - 1.328(TT in °C) n = 42, F = 185, r = 0.948, P 0.00001**
ln SM_{100} in Hrs = 40.002 + 0.0514(AT in °C) - 1.126(TT in °C) n = 42, F = 294, r = 0.967, P 0.00001**

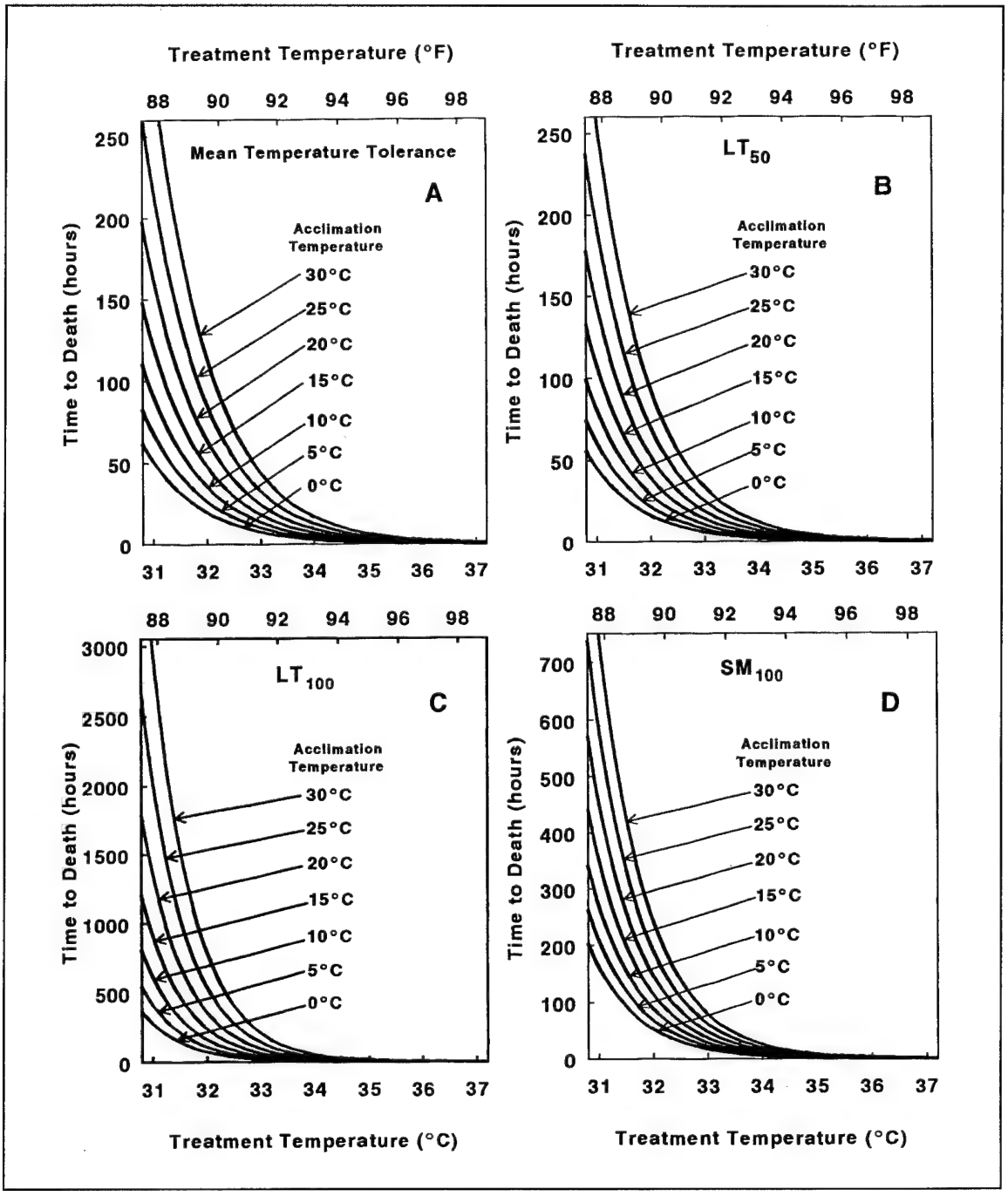

Figure 2. Chronic (incipient) upper lethal thermal tolerance times in zebra mussels, *Dreissena polymorpha*, from the Niagara River estimated from least squares multiple linear regression equations relating tolerance time to acclimation temperature and treatment temperature (Table 6). The vertical axis of all graphs is tolerance time in hours survived at lethal temperatures indicated on the horizontal axis in °C. Individual regression lines are for mussels acclimated to temperatures ranging from 0 to 30 °C. Thermal tolerance times are expressed as (A) mean time to death, (B) LT50 values, estimated time for 50-percent sample mortality, (C) LT100 values, estimated time for near 100-percent sample mortality and (D) SM100 values, actual time of 100-percent sample mortality

4 Discussion

Jenner and Janssen-Mommen (1992) have shown that zebra mussels acclimated to ambient water temperatures in the Netherlands have a chronic upper temperature tolerance of less than 10 min at 36 °C, increasing to 1.5 hr at 33 °C. Mean tolerance times of North American zebra mussels from Lakes Erie and St. Clair exposed to 30 °C varied between 4.74 days when specimens were acclimated to 2.5 °C and 3.96 days when they were acclimated to 25 °C (Iwanyzki and McCauley 1992). At a treatment temperature of 33 °C, these values declined to 0.22 and 17.5 hr in mussels acclimated to 2.5 and 25 °C, respectively, and further declined to 0.17 and 0.65 hr, respectively, when 2.5 and 25 °C-acclimated mussels were exposed to 36 °C (Iwanyzki and McCauley 1992). The mean incipient temperature tolerance times recorded by Iwanyzki and McCauley (1992) for North American zebra mussels were higher than those reported for 100-percent mortality in zebra mussels from northern Europe (Jenner and Janssen-Mommen 1992), even though Iwanyzki and McCauley (1992) reported mean values which underestimate the duration of exposure required to achieve 100-percent mortality. However, their values are similar to those quoted from other, unpublished sources (as cited in Iwanyzki and McCauley 1992) for North American zebra mussel populations from Lake Erie and the Rybinskoye Vodokhranifishche Reservoir in northwestern Russia.

The values for chronic upper lethal temperature tolerance presented in Chapter 3 are also indicative of a much higher level of thermal tolerance in North American zebra mussels than reported for this species in northern Europe by Jenner and Janssen-Mommen (1992), who found that a 100-percent kill of zebra mussels can be achieved within a 1.5 hr exposure to 33 °C. The regression equation for 100-percent sample mortality (i.e., SM_{100}) predicts that a zebra mussel would tolerate 33 °C for 17 to 80 hr over an acclimation range of 0 to 30 °C (Table 6, Figure 2D), a tolerance 11 to 53 times greater than that reported for northern European mussels. Regression equations relating thermal tolerance times to treatment temperature for North American zebra mussels from Lakes Erie and St. Clair indicate that on exposure to 33 °C the mean thermal tolerance times of zebra mussels acclimated to 15, 20, and 25 °C would be 2.8, 6.8, and 10.2 hr, respectively (Iwanyzki and McCauley 1992). Corresponding values for a 15-mm-SL zebra mussel estimated from our regression equation relating tolerance time to acclimation temperature, treatment temperature, and SL (Table 6, Figure 2A) would be 18.5, 24.8, and

33.2 hr, respectively, 6.6, 3.6, and 3.3 times greater than those previously reported. In addition, Iwanyzki and McCauley (1992) reported that 30 °C was the lowest incipient upper lethal temperature inducing death in North American zebra mussels with tolerated exposures ranging from 64 hr in 20 °C-acclimated individuals to 114 hr in 2.5 °C-acclimated individuals. In contrast, the present authors recorded minimal short-term mortality in mussels maintained at this temperature and determined the chronic upper lethal temperature to be 31 °C with mean tolerated exposure times ranging from 52 to 292 hr, depending on acclimation temperature (Figures 1 and 2A).

The basis for the difference in thermal tolerance between these two groups of mussels from the Great Lakes is difficult to ascertain. However, Iwanyzki and McCauley (1992) did not let experimental specimens byssally attach prior to thermal tolerance determinations. McMahon, Shipman, and Long (1992) noted that lack of byssal attachment reduces the tolerance of zebra mussels to nonoxidizing biocides; thus, lack of byssal attachment by experimental individuals in previous determinations may have resulted in a reduction in recorded thermal tolerance values. Iwanyzki and McCauley (1992) also introduced test individuals directly from holding media at the acclimation temperature into media at the test temperature, perhaps subjecting them to a thermal shock which could have effected a reduction in subsequently recorded thermal tolerance times. In contrast, for the study described herein, tank temperature was increased at a rate of 1 °C/10 min from the acclimation temperature to the test temperature to avoid any thermal shock to test specimens that could be induced by an instantaneous temperature change. Such a slow increase in temperature from the acclimation to treatment temperature also better reflects the strategy with which chronic thermal treatment would be applied in industrial or steam-electric power facilities, with temperature being relatively slowly increased from the normal operating (i.e., acclimation) level to the lethal treatment level through partial recirculation of heated effluents (Neuhauser et al. 1993).

The thermal tolerance of *D. polymorpha*, whether measured as incipient upper lethal temperature limit (this study) or acute upper lethal temperature (McMahon et al. 1993), is lower than that of other common North American macrofouling bivalve species. At 32.2 °C, 95-percent mortality was induced in specimens of the marine, macrofouling, blue mussel, *Mytilus edulis* L., within 23 hr, with tolerance time decreasing to 0.23 hr at 40.5 °C (Stock and Del La Parra 1993). In another study, maintenance of blue mussels at 35 °C (95 °F) for 1 hr induced 56-percent sample mortality and, at 40 °C, 100-percent sample mortality was induced within 0.33 hr (Johnson et al. 1983). The short-term upper thermal limit of the Atlantic or American oyster, *Crassostrea virginica* Gmelin, is 48.5 °C (Sellers and Stanley 1989) a value much higher than the 38 °C recorded in this study for *D. polymorpha*. The freshwater, macrofouling Asian clam, *Corbicula fluminea* (Müller), is also considerably more thermally tolerant than *D. polymorpha*. The instantaneous upper lethal temperature of Asian clams is approximately 44 °C in individuals acclimated to 32 °C, with the lowest lethal temperature being 30 °C in 5 °C-acclimated clams which survived exposure to this temperature for less than

4 to 7 hr (Mattice 1979). In *C. fluminea*, 36 °C is the minimum long-term chronic upper lethal temperature (McMahon and Williams 1986), while the present study indicates that it is 31 °C in *D. polymorpha*. The greatly elevated upper thermal limits of *C. fluminea* relative to *D. polymorpha* reflects the Asian clam's endemic distribution in tropical and subtropical areas of southeast Asia (Morton 1979).

The reduced thermal tolerance of *D. polymorpha* relative to other North American biofouling species makes it particularly susceptible to chronic thermal mitigation treatment. Mitigation treatment with temperatures greater than or equal to 34 °C (93 °F) could induce near 100-percent kills of zebra mussel infestations within 6 to 26 hr depending on the prior acclimation/operating temperature experience of mussel infestations (Figure 2D). Below treatment temperatures of 34 °C, the exposure times required for near 100-percent mussel kills become too extended (17 to 80 hr depending on acclimation temperature, Figure 2D) to be economically applied in most industrial or electric generating facilities (Electric Power Research Institute 1992; Claudi and Mackie 1993; Neuhauser et al. 1993). At treatment temperatures ranging between 34 and 37 °C (93 to 99 °F), times for 100-percent kills of zebra mussels are short enough (Figure 2D) to be cost-effective, application temperatures are low enough to prevent major loss of production or excessive equipment wear and/or malfunction, and discharge temperatures are likely to be low enough to meet the discharge temperature restrictions of state and/or national regulatory agencies (Electric Power Research Institute 1992; Neuhauser et al. 1993).

Use of acute thermal mitigation strategies for zebra mussels in which the temperature of a raw system is increased until the instantaneous upper lethal temperature of the zebra mussel is exceeded, while allowing for shorter durations of thermal treatment, requires subjecting raw water systems to higher water temperatures. Thus, at an acclimation temperature of 20 °C (68 °F) and a water heating rate of 1 °C/5 min (a heating rate achievable by thermal backwashing in electric power stations, see Neuhauser et al. 1993), a temperature of 42.3 °C (108 °F) would have to be achieved to induce a 100-percent kill of zebra mussel infestations over a total water heating period of approximately 2 hr (based on SM_{100} values, McMahon et al. 1993), while a chronic thermal mitigation strategy would produce 100-percent kills of zebra mussels within 15.6 to 0.53 hr if applied at 34 to 37 °C (93 to 99 °F) (Figure 2D). Thus, acute thermal mitigation of zebra mussel infestations in entire raw water systems may generate unacceptably high equipment operating and discharge water temperatures, making chronic thermal treatment a more applicable strategy for system-wide mitigation of zebra mussel fouling. In contrast, acute thermal treatments may be most efficacious for treatment of off-line components in which elevated water temperatures would be difficult to maintain for prolonged periods (e.g., use of steam injection to increase to lethal limits the water temperature in mussel-fouled, off-line intake embayments, Kovalak 1993) or for on-line treatment of individual components or sections of raw water systems (e.g., treatment of the service water system only, treatment of only one of several units, or treatment of individual heat exchangers) whose

high treatment discharge water temperatures could be tempered by mixing with untreated waters in the discharge channel.

The data presented here (Figures 2 A-D) and in previous papers (Iwanyzki and McCauley 1992, McMahon et al. 1993) clearly demonstrate that previous temperature acclimation greatly affects both the acute and chronic thermal tolerance of zebra mussels. Thus, regardless of the thermal treatment strategy employed, higher water temperatures will be required to achieve 100-percent mitigation of zebra mussel infestations during summer months (Figures 1 and 2, McMahon et al. 1993). Thus, with either chronic or acute thermal treatment strategies, initiating treatments during periods when water temperatures are below maximal summer levels may significantly reduce both the amount of time required to apply the treatment and/or the temperature required to achieve 100-percent mitigation of mussel fouling. Also of importance is the fact that smaller zebra mussels have greater thermal tolerance times than larger mussels, thus infestations consisting primarily of smaller individuals, which is the usual case if a raw water system is subjected to annual or biannual mitigation treatments, will require higher and/or longer exposures to lethal temperatures to induce near 100-percent mussel kills.

5 Conclusions

The data presented strongly suggest that chronic thermal treatment can be a highly effective means of mitigating zebra mussel fouling in the raw water systems of facilities such as steam-electric power stations which produce heated effluents and are capable of partial recirculation of those effluents into intake structures. Chronic thermal treatment may be particularly applicable for mitigation of *D. polymorpha* fouling as this species appears to have the lowest level of thermal tolerance among common North American macrofouling bivalve species. Based on the model equations relating intake water temperature and treatment temperature to tolerance times in Table 6 and Figure 2, efficacious chronic thermal mitigation of zebra mussel infestations resulting in 100-percent mitigation of fouling within less than a 24-hr treatment period could occur at treatment temperatures of 34 °C (93 °F) and above during summer months when intake water temperatures are equal to or greater than 20 °C (68 °F), and during winter months at 33 °C (91 °F) when water temperatures are 5 °C (41 °F) or below (Figure 2D). Because the thermal tolerance time of zebra mussels exponentially decreases with increased treatment temperature (this study; Iwanyzki and McCauley 1992; Jenner and Janssen-Mommen 1992), the duration of application of a chronic thermal treatment will be greatly reduced with even small increases in treatment temperature (Figure 2), reducing the productivity losses and equipment wear associated with system operation at above-normal temperatures for prolonged periods.

This research has indicated that the chronic thermal tolerance levels of North American zebra mussels are at least an order of magnitude greater than those reported for this species in Northern Europe (Jenner and Janssen-Mommen 1992). While available data are sparse and plagued by incongruent protocols for measurement of thermal tolerance (see Chapter 4), our results do appear to suggest that North American populations of *D. polymorpha* may have been introduced to the Great Lakes from a zebra mussel population in the southern portion of this species' present European range, where elevated ambient water temperatures may have selected for a more thermally tolerant physiological race than exists in the cooler freshwaters of northern Europe. Further evidence of a southern European origin for the race of zebra mussels introduced into the Great Lakes is the concurrent introduction of a second, dreissenid species *Dreissena bugensis* Andrusov, the "quagga mussel" (May and Marsden 1992, Spidle et al. 1994). *Dreissena polymorpha* is the only

dreissenid species found in the freshwaters of northern Europe (Mackie et al. 1989, Stańczykowska 1977). In contrast, *D. bugensis* is restricted to the Southern Bug and Dnieper Rivers (Spidle et al. 1994, Zhadin 1952), which empty into the Dnieper Estuary on the northern shore of the Black Sea in the Ukraine. At the confluence of both rivers is the Ukrainian city of Nikolayev, a major international shipping port, making the northern shore of the Black Sea, and particularly shipping ports such as Nikolayev and the nearby city of Odessa, the likely source of the two dreissenid species introduced into North America either as veliger larvae transported in ballast water (Mackie et al. 1989) or as adults attached to anchor chains (McMahon, Ussery, and Clarke 1993).

The Black Sea and Southern Bug and Dnieper Rivers are at the most extreme southeastern and likely warmest portion of the distribution range for zebra mussels in Europe (Stańczykowska 1977). Therefore, zebra mussels introduced to North America from this region could have been drawn from a genetically, thermally tolerant population relative to those found in the much cooler waters of northern Europe. In any case, the elevated thermal tolerance of North American zebra mussels demonstrated by this research and that of McMahon et al. (1993) strongly suggests that zebra mussels are likely to extend much further south into the freshwater drainage systems of the United States than has been previously estimated based on available information on the temperature tolerance of northern European zebra mussel populations (Electric Power Research Institute 1992; McMahon 1990, 1992; Strayer 1991). Indeed, *D. polymorpha* has already been reported to have invaded the waters of the lower Mississippi River as far south as New Orleans (Zebra Mussel Information Clearinghouse 1993), where it appears to be successfully reproducing in areas with summer surface water temperatures approaching 30 °C (T. H. Dietz, personal communication). The capacity of north American *D. polymorpha* to survive 30 °C (this study; McMahon et al. 1993) strongly suggests that this species will not be restricted by elevated ambient temperatures in its invasion of North American freshwater drainages in all but the warmest regions of the southwestern United States and Mexico (Water Information Center, Inc. 1973).

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Chronic (i.e., long-term incipient) upper lethal temperatures were determined for zebra mussels (*Dreissena polymorpha*) acclimated to 5, 10, 15, 20, 25, or 30 °C for at least 14 days. Subsamples (n = 25-33) from each acclimation group were exposed to constant temperatures of 31, 32, 33, 34, 35, 36, or 37 °C (±0.1 °C). Mussels were brought from acclimation temperature to test temperature by increasing media temperature at a rate of 1 °C/10 min. After reaching the test temperature, mortality was determined every 15 min for the first 2 hr, every 30 min for the next 3 to 5 hr, and every hr for the next 17 to 21 hr and every 3 to 4 hr thereafter until all individuals had died. Multiple regression analysis indicated that acclimation temperature, exposure temperature, and mussel shell length (SL) were significantly correlated ($P < 0.00001$) with the natural log of thermal tolerance time. Tolerance time increased with increasing acclimation temperature and decreasing SL and decreased with increased exposure temperature. LT_{50} values (i.e., estimated time for 50-percent sample mortality) ranged from <1 hr at 37 °C regardless of acclimation temperature to 456 hr in 25 °C-acclimated individuals at 31 °C. Tolerance times were much greater than reported for northern European zebra mussels (100-percent kill within 3 hr at 32 °C compared with 54 to 399 hr dependent onacclimation temperature). Multiple regression analysis indicated that the

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natural logarithm of either LT_{50}, LT_{100}, or SM_{100} (actual hours for 100-percent sample mortality) was significantly affected by both acclimation temperature and exposure temperature ($P < 0.00001$), increasing with increasing acclimation temperature and exponentially decreasing with increasing test temperature. Mathematical models are presented which allow prediction of the duration of exposure to lethal temperatures to induce mortality in zebra mussel infestations based on the temperature of thermal treatment, and the prior operating temperature experience of mussel infestations.